‘‘Design , Development and Evaluation of Phytosomes.’’

A SYNOPSIS

Submitted for the consideration of

Registration for the Degree of *Doctor of Philosophy*

In the Faculty of Pharmaceutical Sciences and Technology,

By

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Under the supervision of

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PROFORMA FOR REGISTRATION OF TOPIC FOR DESSERTATION

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3.	**COURSE OF STUDY AND SUBJECT**	**:**	PhD (PHARMACEUTICS)
4.	**DATE OF ADMISSION TO THE COURSE**	**:**	
5.	**TITLE OF RESEARCH TOPIC**	**:**	**Design , Development and Evaluation of Phytosomes.**

6.	**Objective of Research Topics:**
6.1	**Main Objective:** 1. Preformulation study of phytosomes by Quality by Design(QbD Approach). 2. Formulation Development and evalution of phytosomes . 3. To study the In-vitro and In- vivo (IVIV) correlationship of phytosmomes. 4. To study the stability of prepared phytosomes as per ICH guidelines.

6.2	**Hypothesis:** In the pharmaceutical field, liposomes have long been of great interest by offering a promising way for both systemic and locally acting drugs used for therapeutic applications in humans and animals. A liposome encapsulates a region on aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the phytosomes contents. Many hundreds of drugs, including anticancer and antimicrobial agents, chelating agents, peptide hormones, enzymes, proteins, vaccines, and genetic materials, have been incorporated into the aqueous or lipid phases of phytosomes, with various sizes, compositions, and other characteristics, to provide selective delivery to the target site for in vivo application. As a result of the great potential of phytosomes in the area of drug delivery, the potent drugs are administered in its free forms,that can otherwise produce severe side-effects and their encapsulation significantly diminishes the unwanted properties. As a drug delivery system,Phytosomes are playing acrucial role to improve their therapeutics, reduce their toxicity and enhance the efficacy of drugs for the treatment of diseases.

7. REVIEW OF LITERATURE:

1) Asmita Singh, Prerana Vengurlekar, Sudha Rathod Liposomal formulations have been successfully used in the treatment of a number of dermatological diseases. Various synthetic as well as herbal drugs are incorporated into liposome to improve its efficacy. Incorporation of herbal extract into liposome reduces side effects which are associated with the synthetic ones. *Azadirachta indica* leaves possesse good anti bacterial activity, confirming the great potential of bioactive compounds of neem. Among aqueous extract and alcoholic extract, alcoholic leaf extracts of A. indica were found to be more active towards the bacterial species. Hence, this extract was incorporated into liposomes to enhance its activity in skin delivery. The work has converted age old miraculous herb into nanotechnology based formulations i.e. liposomes. An attempt has been made to prepare liposomal Neem gel for topical use for anti-microbial activity. Methods: Methanolic Neem Extract (MeNE) was incorporated into liposomes by thin film hydration method. The batch having lipid ratio i.e. Soya lecithin: Cholesterol (4:1); MeNE concentration 80 mg with entrapment efficiency 69.52 ±1.9% was finalized.[10]

2) Javad Asili1, Navid Mosallaei, Ali Shaterzadeh, Bizhan Malaekeh-Nikouei *Platycladus orientalis* or *Thuja orientalis* is a native plant of Iran. Different parts of the plant are used in the treatment of various diseases such as: gout, rheumatoid arthritis, common cold, cough,bronchitis, asthma, high blood pressure and hormonal disorders like hirsutism and baldness. Also, various organs of this species have been used as appetizer. The purpose of this study was to prepare and characterize liposomal formulations that contain methanol extract of aerial parts of *P. orientalis* for hirsutism treatment.[7]

3) Muntamon Utamo, Hathairat Rimkeeree, Vichai Haruthaithanasa The emblica extraction was conducted using Soxhlet extraction method with ethyl acetate as a solvent. The gallic acid content of emblica extract was found to be 300.30 mg GAE g-1 and antioxidant activity(SC50) 0.042±0.004 mg ml-1. The emblica liposome was prepared from Chloroform film consisted of SPC : Tween80 : DA in the ratio 84: 16: 2.5 M weight ratio and 0.2 gL-1 of emblica extract. The particle size was reduced using Microfluidizer at 16,000 psi for 3 cycles. The freeze dried powder showed that liposomes were pale yellow with pH values of 5.74. The particle size of emblica liposomes was nanoparticle and result of TEM equal 50-100 nm, on the surface charge -59.1±1.08 mV and index distribution of the trapped particles 0.236±0.03 PDI. Liposomes

prepared by large unilamellar vesicles (LUV). The gallic acid content of emblica liposomes was found to be 11.04 mg g-1. Antioxidation activity (SC50) was 3.05±0.82 mg ml-1. The entrapment efficiency of emblica liposome was decreased when the time increased.

4) Eskandar Moghimipour , Nasrin Aghel, Ali Zarei Mahmoudabadi , Zahra Ramezani , Somayeh Handali The increased incidence of fungal resistance has necessitated the need tosearch for new antifungal agents. The main objectives of the present study were to investigate the effectiveness of the essential oil of *Eucalyptus camaldulensis* on dermatophytes growth and to formulate and characterize a liposomal gel loaded with the essential oil. The essential oil extracted from the leaves of *E. camaldulensis* was analyzed by GC-MS. The antifungal activity of this essential oil was determined against Microsporum canis, M. gypseum, Trichophyton rubrum and T. verrucosum, using the well diffusion method. Liposomes were prepared by the freeze-thaw method and evaluation of size distribution was performed using a particle size analyzer. The liposomal gel was prepared using 'hydroxethyl cellulose (HEC) as the gelling agent. The rheologic characteristics were determined by a Brookfield viscometer. The results showed that the minimum inhibitory volume of the essential oil was 0.125 ml and 95 ± 0.57% of the essential oil was successfully entrapped in the liposomes. The main constituents of the essential oil detected by GC-MS were; phenol, 1, 8 cineole, limonene, alcohol, pinene and terpinen. Results of particle size determination showed a wide range from 40.5 to 298 nm for the different formulations. No significant thixotropy was observed in the rheogram of the formulated liposomal gel. Liposomal gel formulation of the essential oil may lead to improved antifungal activity.[11]

5) Swati Patil, Supritha Shetty, Rama Bhide and Shridhar Narayanan *Carica papaya* leaves have been used traditionally to treat indigestion, as a vermifuge. *Carica papaya* leaves have also been shown to possess anti-tumor and immunomodulatory effects. The current study aims at determining the effect of *Carica papaya* leaves aqueous extract in increasing the platelet count in thrombocytopenic rat model. Aqueous extract of *Carica papaya* leaves at concentration of 400mg/kg and 800mg/kg were given to cyclophosphamide induced thrombocytopenic rats for a period of fifteen days. Blood was withdrawn at various time intervals to determine the platelet count. Also, the clotting time was determined on the 15th day of the study by capillary method. Carica papaya leaf extract was found to increase the platelet count and also to decrease the

clotting time in rats. The study aims at determining the possible effects of papaya leaves in thrombocytopenia occurring in dengue fever.

6) Jyotsna Kiran Peter, Yashab Kumar, Priyanka Pandey and Harison Masih The study dealt with the antibacterial activity of aqueous, chloroform extract of leaves and aqueous, methanolic extract of seeds of *Carica papaya* var. pusa dwarf through agar well diffusion assay against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli Salmonella typhi*. It was revealed that the aqueous as well as the methanolic extract of seeds were effective to inhibit the bacterial pathogens while in case of chloroform extract of *Carica papaya* leaves did not show any inhibition against the bacteria and the aqueous leaf extract was potent to inhibit them.

7) **Eskandar Moghimipour, Somayeh Handali** Liposomes are polymeric nanoparticles used for drug delivery due to their unique properties. Lipisomes can encapsulate both hydrophobic and hydrophilic drug. liposomes deliver the drugs into cells by fusion or endocytosis mechanisms. In the last few decades, liposomes have been considered as ideal models for mimic biological membranes and also they are suitable carriers for drugs, diagnostics, vaccines, and other bioactive agents. Drugs can be distributed non-specifically throughout the body, lead to death of normal and malignant cells. Entrapment of drugs into liposomes results in increasing circulation life time, protection from the metabolic degradation, enhancement of deposition in the infected tissues and decreased uptake in the kidney, myocardium and brain.

8) Janina Gabrielska, Małgorzata Korzeniowska, Aneta Wojdyło the use of liposomes and erythrocytes membrane as a real membrane models to evaluate the potential benefits of several plants extracts and two flavones in relation to lipid peroxidation. The antioxidant behaviour of the plant extracts from pine (*Pinus silvestris*), hawthorn (*Crataegus oxyacantha* , two extracts: from hawthorn's leaves-l and bark-b), evening primrose (*Oenothena paradoxa* Hudziok – three extracts differ in procyanidins content P1, P2 and P3) and rosemary (*Rosmarinus officinalis*, as a standard for extracts) and flavones of baicalin and rutin have been studied. The results obtained showed that the studied extracts exhibited differentiated, dose-dependent antioxidant activity against phospatidylcholine liposomes (rosemary>pine≈hawthorn-l>hawthorn-b≈P1≈P2≈P3; statistically significant differences were observed between the extracts at $p \leq 0.05$) and erythrocyte membranes (rosemary≥hawthorn-b≈hawthorn-l>P1≈pine>P2≈P3) when the

oxidation was induced by UV-C radiation. They also reduce the oxidation of liposomes and erythrocyte membrane when its oxidation was induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (pine≥P1≈P2≈rosemary≈P3 in the case of liposomes and rosemary>> pine≥P1≈P2≥P3 in a case of erythrocyte). Moreover, the results of the study show that baicalin is characterized by high inhibition ability towards liposome PC peroxidation, as well as towards erythrocyte ghosts, when oxidation was initiated by UV radiation. However, at the same experimental conditions, the inhibitory capacity of rutin was about 7-8 times weaker. The presence of cholesterol in liposome membrane decreased the level of membrane peroxidation but do not influenced on the antioxidant activity of hawthorn extract.[9]

9) Arijit Gandhi , Avik Dutta , Avijit Pal , Paromita Bakshi The effectiveness of any herbal medication is dependent on the delivery of effective level of the therapeutically active compound. But a severe limitation exists in their bioavailability when administered orally or by topical applications. Phytosomes are recently introduced herbal formulations that are better absorbed and as a result produced better bioavailability and actions than the conventional phyto molecules or botanical extracts. Phytosomes are produced by a process whereby the standardized plant extract or its constituents are bound to phospholipids, mainly phosphatidylcholine producing a lipid compatible molecular complex. Phytosome exhibit better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts. The present review represents the recent advances and applications of various standardized herbal extract phytosomes as a tool of drug delivery.[2]

10) Abdalrahim FA Aisha, Amin Malik Shah Abdul Majid and Zhari Ismail1 *Orthosiphon stamineus* is a medicinal herb with remarkable pharmacological properties. However, poor solubility of the active principles limits its medicinal value. This study sought to prepare nano liposomes of OS ethanolic extract in unpurified soybean phospholipids in order to improve its solubility and permeability. OS liposomes were prepared by the conventional film method, and were characterized for solubility, entrapment efficiency, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), particle size and zeta potential, release, absorption in everted rat intestinal sacs, and DPPH scavenging effect.[8]

11) Olga Gortzi , Stavros Lalas , Ioanna Chinou John Tsaknis The antioxidant and antimicrobial activity of methanol or dichloromethane extracts of *O. dictamnus*, produced from wild and organic cultivated specimens, were determined. The Rancimat and malondialdehyde (MDA) by HPLC methods were used to measure the antioxidant action, in comparison with that of the common commercial antioxidants butylated hydroxytoluene (BHT) and α-tocopherol. The extracts that presented high antioxidant activity were encapsulated in liposomes and their antioxidant action was again estimated using differential scanning calorimetry (DSC). Thermaloxidative decomposition of the samples (pure liposomes and encapsulated extracts) and the modification of the main transition temperature for the lipid mixture and the splitting of the calorimetric peak in the presence of the antioxidants were also studied by the DSC method. All extracts showed antioxidant and antimicrobial activities. Their action proved superior to α-tocopherol. The methanol extract of organic cultivated *O. dictamnus* (240 ppm) showed higher activity than butylated hydroxytoluene. After encapsulation in liposomes the antioxidant as well as antimicrobial activities proved to be higher than those of the same extracts in pure form.

12) Mansoori M. A., Agrawal S., Jawade S., Khan M. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilicdrugs and protect them from degradation. Liposomes are microparticulate lipoidal vesicles which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Due to new developments in liposome technology, several liposome-based drug formulations are currently in clinical trial, and recently some of them have been approved for clinical use. Reformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their biodistribution. This review discusses the potential applications of liposomes in drug delivery withexamples of formulations approved for clinical use,their preparation method, targeting, mechanism of formation, liposome component and the problems associated with further exploitation of this drug delivery system.

13) Hemanth kumar Liposomal encapsulation technology is the newest delivery method used by medical researchers to transfer drug that act as healing promoters to definite body organsThis forms of delivery system offers targeted delivery of vital compounds to the body.LET is a state of the art method of sub-miroscopic bubbles called liposomes which encapsulate various drugs.The liposomes form a barrier around their contents that is resistant ro enzymes in mouth

and stomch, digestive juices, alkaline solutions, bile salts and intestinal flora found in human body as well as free radicals.The contents of the liposome s are shielded from degradation and oxidation.This protective phospholipids shield or barrier remains unharmed until the contents of the liposome are delivered to the target organ,gland and system, where the content are utilized natural extracts are generally degraded because of oxidation and other reactions before they delivered to target site.Our research shown that LET of ayurvedic preparations have shown more stability and more efficiency when compared to traditional preparation. Size of liposomes were measured around 85-200nm.[5]

14) **Nayyer Karimi, Babak Ghanbarzadeh, Hamed Hamishehkar** Nutracutical materials usually show less bioactivity than optimal state.Phytosomes are better absorbed and improved bioavailability of bioingredients.They are produced by a process whereby the standardizedplant extract or its constituents are bound to phospholipids, mainly phosphtidylcholine.Infact that they are advanced forms of herbal formulations contains bioactive substance of herb extract such as flavonoids, glycosides, terpenoids and good ability to transition from a hydrophilic into liphophilic environment of the outer cell membrane.It has good bioavailabilty and function than conventional herbal plant extracts .Its shows that effectively to increased the bioavailability of herbal plant extract.[13]

8.Material and Methods

1. Selection of drug and excipients.

2. Procurement of drug and excipients.

3. Preformulation studies:

A) Raw material characterization

- Characterization of drug.
- Characterization of excipients.

B) Structural Characterization

- UV
- FTIR
- DSC
- XRD

C) Physicochemical Characterization

- Solubility studies.
- Loss of drying.
- pH determination.

4. Formulation study

A) Designing by QbD

B) Evaluation of various parameters:

- Vesicle shape.
- Vesicle size by Scanning Electron Microscopy.

- Entrapment efficiency.
- FT-IR
- XR
- IVIV correleationship study.
- Stability studies as per ICH guidelines.

9. CURRENT STATUS OF HERBAL PLANT EXTRACT

1) US Patent (US-005643598a) Method of Skin Care Utlising Liposomes Contaning Scutellaria Extract .

2)US Patent Herbal (US-006312736b1) Herbal Composition to Relieve Pain.

3) US Patent (US20140248332a1) Onion Extract Encapsulated in Liposome.

10. DURATION OF WORK

Sr.	WORK	PERIOD
1	Literature survey	6 Months
2	Procurement of required ingredients	2 Months
3	Characterization of procured ingredients.	4 Months
4	Selection & procurement of drug	8 Months
5	Pre-formulations Study	4 Months
6	Assimilation of ingredient in appropriate dosage form	4 Months
7	Evaluation of dosage form	8 Months
	Total duration of project	**36 Months**

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13) Nayyer Karimi, Babak Ghanbarzadeh, Hamed Hamishehkar., Phytosomes as novel delivery system for Nutracutical materials., **International Journal of Current Microbiology and Applied Sciences**., ISSN:2319-7706, Vol-4, p.n-152-159, 2015.

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